

# NF- $\kappa$ B (p65) protein expression in head and neck tumors and its association with clinicopathological parameters

PRECIOUS BARNES<sup>1</sup>, ABRAHAM MENSAH<sup>2</sup>, LEONARD DERKYI-KWARTENG<sup>3</sup>, ERNEST ADANKWA<sup>4</sup>, ELVIS AGBO<sup>5</sup>, EWURA SEIDU YAHAYA<sup>6</sup>, BENJAMIN AMOANI<sup>7</sup>, EMMANUEL GUSTAV IMBEAH<sup>2</sup>, GEORGE ADJEI<sup>8</sup>, PATRICK KAFUI AKAKPO<sup>3</sup>, FAUSTINA HALM-LAI<sup>2</sup>, KWABENA DANKWA<sup>2</sup>, SAMUEL VICTOR NUVOR<sup>2</sup>, ROLAND OSEI SAAHENE<sup>2</sup> and DORCAS OBIRI-YEBOAH<sup>2</sup>

<sup>1</sup>Department of Chemical Pathology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast 32440, Ghana; <sup>2</sup>Department of Microbiology and Immunology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast 32440, Ghana; <sup>3</sup>Department of Pathology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast 32440, Ghana; <sup>4</sup>Department of Medical Diagnostics, Kwame Nkrumah University of Science and Technology, Kumasi 1279, Ghana; <sup>5</sup>Department of Human Anatomy, Histology and Embryology, College of Medicine, Jिंगgangshan University, Ji'an, Jiangxi 343000, P.R. China; <sup>6</sup>Department of Pharmacology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast 32440, Ghana; <sup>7</sup>Department of Biomedical Sciences, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast 32440, Ghana; <sup>8</sup>Department of Community Medicine, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast 32440, Ghana

Received October 7, 2024; Accepted February 14, 2025

DOI: 10.3892/wasj.2025.330

**Abstract.** Head and neck tumors (HNTs) are a diverse group of tumors that develop due to a variety of genetic and environmental risk factors with varying incidence rates. Nuclear factor  $\kappa$ B (NF- $\kappa$ B), is a transcription factor that has been linked to malignant tumors and chronic inflammation. However, there is a paucity of available data globally on NF- $\kappa$ B (p65) in HNTs. The present study evaluated the association between clinicopathological features of patients with HNT and NF- $\kappa$ B (p65) protein expression. Immunohistochemistry was used to examine NF- $\kappa$ B (p65) expression in 112 HNT tissues comprising of 62 benign and 50 malignant tumors. The Chi-square test and Fisher's exact test were employed to analyze the association between NF- $\kappa$ B (p65) protein expression patterns and the clinicopathological features of the patients. NF- $\kappa$ B (p65) protein was expressed in 58.9% of head and neck tumors evaluated. There was a significant association between NF- $\kappa$ B (p65), sex and tumor type. There was a high NF- $\kappa$ B (p65) expression in malignant tumors, with its

expression in malignant tumors being 2-fold higher than that in benign tumors. NF- $\kappa$ B (p65) expression in malignant tumors was associated with the male sex and tumor node metastasis (TNM) stage. On the whole, the present study emphasizes that NF- $\kappa$ B (p65) protein expression levels may indeed be controlled by sex and TNM stage, with a higher NF- $\kappa$ B (p65) activity observed in male patients with HNTs. Therefore, it may be explored as a potential prognostic biomarker and therapeutic target.

## Introduction

Head and neck tumors (HNTs) are a diverse group of tumors that develop due to a variety of genetic and environmental risk factors (1). HNTs commonly occur in the larynx, oral cavity, sinonasal tract and nasopharynx (2). Risk factors, such as smoking, excessive alcohol consumption, and long-term exposure to ultraviolet radiation, viral infections and asbestos have been documented to lead to the development of HNTs (1). In developing countries, tobacco and alcohol, which contribute to inflammation, are responsible for >90% of HNT cases. Moreover, in developed countries, human papillomavirus infection with oncogenic features accounts for >70% of cases (3,4). Although recent progress has been made in the diagnosis and treatment of head and neck cancer (HNC), there have been minimal improvements in life expectancy over the past few decades (5). Surgery, radiation therapy and chemotherapy are the currently available treatment options (6). However, local recurrence and metastasis occur in approximately half of patients with HNC (7).

---

*Correspondence to:* Dr Roland Osei Saahene, Department of Microbiology and Immunology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Sixth Close, CNC V212, Cape Coast 32440, Ghana  
E-mail: roland.saahene@ucc.edu.gh

*Key words:* NF- $\kappa$ B (p65), head and neck tumor, sex, tumor node metastasis

According to Owusu-Afriyie *et al* (8), the incidence of HNC varies greatly across sub-Saharan Africa, with the incidence in Ghana being 0.8 per 100,000 individuals, compared to 11.1 per 100,000 individuals in South Africa. Biomarkers including p53, epidermal growth factor receptor, p16 and cyclin-D1 have been studied in Ghana to assess their prognostic significance (8-10). However, alternative therapeutic markers remain unexplored.

Nuclear factor  $\kappa$ B (NF- $\kappa$ B), a transcription factor with five family members, namely p105/p50 (NF- $\kappa$ B1), p100/p52 (NF- $\kappa$ B2), p65 (RELA), c-REL and RELB, has been linked to chronic inflammation and malignancies, including HNC. The increased expression of NF- $\kappa$ B target genes has been shown to be associated with tumorigenesis (11,12). NF- $\kappa$ B is normally inactive in the cytosol, bound to the inhibitor of  $\kappa$ B (I $\kappa$ B). The I $\kappa$ B kinase (IKK) stimuli-mediated phosphorylation degrades I $\kappa$ B, freeing NF- $\kappa$ B to translocate to the nucleus to perform transcriptional functions and modulate biological roles (13). Studies have shown that NF- $\kappa$ B activation is a key phenomenon that contributes to tumorigenesis and the survival of cancer cells, as observed in pancreatic and breast cancer (14,15). While typically regulated by negative feedback loops, there is a malfunction in NF- $\kappa$ B activity in cancer cells due to mutations or chronic exposure to activating stimuli, leading to its overexpression (13,16). TNF- $\alpha$ , IL-1 and IL-17 produced by immune cells infiltrating the gastrointestinal mucosa can increase NF- $\kappa$ B activity, thereby increasing risk of colon cancer (17). The activation of the NF- $\kappa$ B pathway also promotes metastasis by regulating epithelial-mesenchymal transition (18) and matrix metalloproteinases-9 (19). The increased expression of NF- $\kappa$ B (p65) has been demonstrated to be associated with tumor grade in breast cancer (20). Currently, the clinical value of NF- $\kappa$ B (p65) in tumor specimens is unclear and remains underexplored. Despite advancements being made in cancer research in Africa, including Ghana, to the best of our knowledge, no study available to date has explored the prognostic value of NF- $\kappa$ B (p65) in HNTs. The present study thus aimed to fill this gap by evaluating the prognostic relevance of NF- $\kappa$ B (p65) in patients with HNTs at the Cape Coast Teaching Hospital (CCTH) in Cape Coast, Ghana.

## Patients and methods

### *Study design, ethical approval and sample collection.*

The present cross-sectional study was approved by the Institutional Review Board of CCTH (Approval no. CCTHERC/EC/2023/183). Laboratory analyses were conducted at the Kumasi Center for Collaborative Research (KCCR), Kumasi, Ghana. The inclusion criteria were patients with histologically confirmed HNTs, available archived formalin-fixed paraffin-embedded (FFPE) tissues and complete medical records. The exclusion criteria were patients with a history of no preoperative chemotherapy and no concurrent cancers from 2017 to 2022. The patients who qualified were purposively sampled at the ACT Pathology Consult, the consulting pathology firm of CCTH. Clinicopathological data including age, sex, tumor location, type of biopsy, type of tumor, perineural invasion, tumor node metastasis (TNM), laterality, tumor grade and lympho-vascular invasion were collected from the laboratory request

forms of the patients. Hematoxylin and eosin staining was used to confirm the pathological classification of the HNTs prior to the selection of the tissues. A total of 112 tumor tissues were selected for analysis.

*Immunohistochemistry.* FFPE sections (4- $\mu$ m-thick) were attached to positively charged slides. These portions underwent several preparatory procedures, such as deparaffinization and rehydration with xylene (two changes for 10 min each and various ethanol concentrations (100, 90 and 70% for 3 min each), respectively. A Biocare decloaking chamber (Biocare Medical System, Inc.) set at 95° for 30 min was used for antigen retrieval using Novocastra buffer (pH 9.0) (lot no. 6093858, Leica Microsystems, Ltd.), followed by a 35-min cooling phase in the same buffer and washing with reaction buffer from Ventana (lot no. H18324, Ventana Medical Systems, Inc.).

A protein blocker (Biocare Medical System, Inc.) and 3% hydrogen peroxide were used to inhibit non-specific protein binding and hydrogen peroxidase in the tissues. The tissues were then exposed to appropriately diluted (1:400) NF- $\kappa$ B (p65) rabbit polyclonal antibody IgG (cat. no. 10745-1-AP, Proteintech Group, Inc.) for 2 h at room temperature. The tissues were then rinsed and treated with MACH 1 Universal horse radish peroxidase (HRP) polymer, as a secondary antibody (lot no. 092822A, Biocare Medical System, Inc.) at room temperature for 45 min (no dilution was done). 3,3-Diaminobenzidine (DAB) chromogen (lot no. 010722A-4, Biocare Medical System, Inc.) and substrate (lot no. 071422A-2, Biocare Medical System, Inc.) were added following a second round of washing, and Mayer's hematoxylin (Abcam) was employed for counterstaining at room temperature for 1-2 min. Before mounting the dyed tissues with coverslips using para-mount, the tissues were dehydrated and cleared. Of note, two pathologists then used an Opto-Edu brand [Opto-Edu (Beijing) Co., Ltd.] digital microscope to analyze the slides and read the protein expression of NF- $\kappa$ B (p65) in the HNTs. A brownish-yellow colour observed in the cytoplasm was regarded as positive staining. The semi-quantitative approach, which combines the staining intensity (0, no staining; 1, mild staining; 2, moderate staining; and 3, strong staining) and the percentage of the tumors stained (0, negative; 1, <25%; 2, 25-50%; 3, 51-75%; 4, 76-100%), were used to calculate the final cytoplasmic stain of NF- $\kappa$ B (p65), as previously described (21). Tissues with the product of staining intensity and percentage scores of 0-6 were considered as having a low expression and those with scores of 7-12 were considered as having a high expression. At a magnification of x400, three to four images were obtained.

*Statistical analysis.* Categorical variables are expressed as frequencies and percentages. Pearson's Chi-square test and Fisher's exact test were used to determine the associations between clinicopathological variables using SPSS version 25 software (IBM Corp.). P-values <0.05 were considered to indicate statistically significant differences. Logistic regression analysis was conducted for the associated variables and diagnostic performance was assessed using receiver operation characteristic analysis with XLstat (<https://www.xlstat.com/en/>).

Table I. Association between NF-κB (p65) status and characteristics of patients with head and neck tumors.

Variables, n (%)	Total (n=112)	NF-κB (p65) expression status		χ <sup>2</sup> /Fisher's	P-value
		Low (n=64)	High (n=48)		
Sex				7.146	<b>0.013</b>
Male	56 (50.0)	25 (44.6)	31 (55.4)		
Female	56 (50.0)	39 (69.6)	17 (30.4)		
Age group, years				1.386	0.500
1-29	30 (26.8)	19 (29.7)	11 (22.9)		
30-57	55 (49.1)	32 (50.0)	23 (47.9)		
58-86	27 (24.1)	13 (20.3)	14 (29.2)		
Types of biopsy				3.707 <sup>a</sup>	0.670 <sup>a</sup>
Excision	54 (48.2)	34 (63.0)	20 (37.0)		
Incision biopsy	46 (41.1)	24 (52.2)	22 (47.8)		
Punch biopsy	8 (7.1)	4 (50.0)	4 (50.0)		
Free needle core biopsy	2 (1.8)	1 (50.0)	1 (50.0)		
Curettage	1 (0.9)	1 (100.0)	0 (0)		
Ultrasound guided core biopsy	1 (0.9)	0 (0)	1 (100.0)		
Type of tumor				10.839	<b>0.001</b>
Benign	62 (55.4)	44 (71.0)	18 (29.0)		
Malignant	50 (44.6)	20 (40.0)	30 (60.0)		
Tumor location				15.600 <sup>a</sup>	0.099 <sup>a</sup>
Ear	6 (5.4)	5 (83.3)	1 (16.7)		
Oral cavity	10 (8.9)	5 (50.0)	5 (50.0)		
Salivary	7 (6.3)	3 (42.9)	4 (57.1)		
Eye	5 (4.5)	4 (80.0)	1 (20.0)		
Face	7 (6.3)	7 (100.0)	0 (0)		
Larynx	16 (14.3)	7 (43.8)	9 (56.3)		
Mandible	9 (8.0)	6 (66.7)	3 (33.3)		
Maxillary region	7 (6.3)	3 (42.9)	4 (57.1)		
Nasal cavity	24 (21.4)	16 (66.7)	8 (33.3)		
Nasopharynx	13 (11.6)	4 (30.8)	9 (69.2)		
Neck	8 (7.1)	4 (50.0)	4 (50.0)		
Laterality				1.317	0.518
Left	32 (28.6)	21 (65.6)	11 (34.4)		
Right	28 (25.0)	15 (53.6)	13 (46.4)		
N/A	52 (46.4)	28 (53.8)	24 (46.2)		

Data were analyzed using the Chi-squared test or <sup>a</sup>Fisher's exact test. Values in bold font indicate statistically significant differences (P<0.05). N/A, not applicable.

## Results

*Characteristics of patients with HNTs.* The present study utilized FFPE tissue blocks from 112 patients with various HNTs, aged between 1 and 86 years. The average age of the patients was 42.71±18.85 years. There was an equal distribution of male [56 (50%)] and female [56 (50%)] participants. The analysis of the age distribution revealed that 30 patients (26.8%) were of 1-29 years of age, 55 patients (49.1%) of 30-57 years of age and 27 (24.1) of 58-86 years of age. As regards biopsy types, 54 cases (48.2%) were excisional biopsies, 46 cases (41.1%) were incisional biopsies, 8 cases (7.1%)

were punch biopsies and 2 cases (1.8%) were free needle core biopsies. Additionally, there was an equal representation of curettage and ultrasound-guided core biopsies (n=1; 0.9%). Of the 112 tumors analyzed, 62 (55.4%) were benign and 50 (44.6%) were malignant. The majority of the tumors were located in the nasal cavity 24 (21.4%) and the larynx 16 (14.3%). Other tumor sites included the ear [6 (5.4%)], oral cavity [10 (8.9)], salivary gland [7 (6.3%)], eye [5 (4.5%)], face [7 (6.3%)], mandible [9 (8.0%)], maxillary region [7 (6.3%)], nasopharynx [13 (11.6%)] and neck [8 (7.1%)]. Laterality data revealed that 52 cases (46.4%) had no specific side designation, while 32 cases (28.6%) were from the left side and 28

Table II. Logistic regression analysis of NF- $\kappa$ B (p65) expression vs. sex and type of tumor.

Variables, n (%)	Total (n=112)	NF- $\kappa$ B (p65) status		OR (95% CI)	P-value
		Low (n=64)	High (n=48)		
Sex					0.008
Male	56 (50.0)	25 (44.6)	31 (55.4)	1	
Female	56 (50.0)	39 (69.6)	17 (30.4)	0.352 (0.162-0.764)	
Type of tumor					0.001
Benign	62 (55.4)	44 (71.0)	18 (29.0)	1	
Malignant	50 (44.6)	20 (40.0)	30 (60.0)	0.273 (0.124-0.600)	

OR, odds ratio; CI, confidence interval.

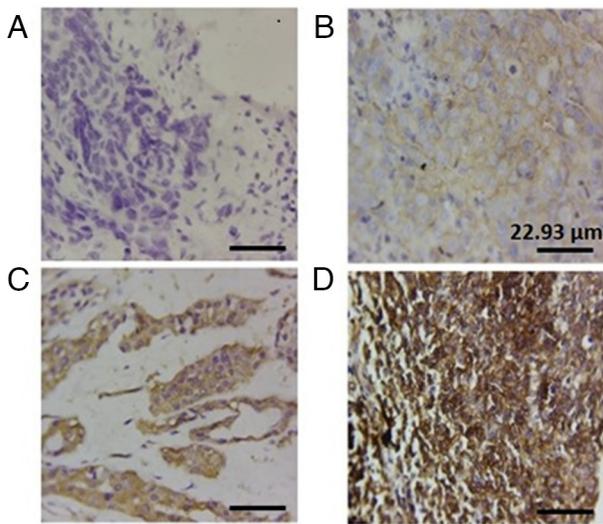


Figure 1. Immunohistochemistry images of various malignant head and neck tumors. (A) Negative staining. (B) Mild staining. (C) Moderate staining. (D) Strong staining. Magnification, x400.

cases (25.0%) were from the right side. The detailed patient characteristics are presented in Table I.

*NF- $\kappa$ B (p65) protein expression in HNTs, as demonstrated using immunohistochemistry.* NF- $\kappa$ B (p65) protein expression was observed exclusively in the cytoplasm, as depicted in Fig. 1. In the benign tumors, NF- $\kappa$ B (p65) expression analysis revealed that 18 (29%) of the tumors exhibited a high expression, while 44 (71%) exhibited a low expression. Conversely, immunostaining in the malignant tumors demonstrated that 30 (60%) of the tissues exhibited a high NF- $\kappa$ B (p65) expression, whereas 20 cases (40%) exhibited a low expression, as detailed in Table I.

*Association of NF- $\kappa$ B (p65) expression with clinicopathological characteristics of patients with HNTs.* Despite the equal number of males and females, there was a significant association between NF- $\kappa$ B (p65) expression and sex ( $P=0.013$ ), with males exhibiting higher levels of NF- $\kappa$ B (p65) protein expression compared to females ( $n=31, 55.4\%$  vs.  $n=17, 30.4\%$ ). Conversely, a low expression of NF- $\kappa$ B (p65) was

more prevalent in female tumors ( $n=39; 69.6\%$ ) compared to male tumors 25 (44.6%). Additionally, a significant association ( $P=0.001$ ) was observed between tumor type (benign or malignant) and NF- $\kappa$ B (p65) expression. A high protein expression of NF- $\kappa$ B (p65) was twice as common in malignant tumors compared to benign tumors ( $n=30, 60.0\%$  vs.  $n=18, 29.0\%$ ). However, NF- $\kappa$ B (p65) protein expression in HNTs was not significantly associated with age, type of biopsy, location of tumor and laterality (Table I).

Logistic regression analysis revealed a significant decrease in odds for NF- $\kappa$ B (p65) expression when comparing sex [odds ratio (OR), 0.352; 95% confidence interval (CI), 0.162-0.764;  $P$ -value=0.008] and tumor type (OR, 0.273, 95% CI, 0.124-0.600;  $P$ -value=0.001) (Table II). When the data were stratified to examine the associations between benign tumors and NF- $\kappa$ B (p65) expression (Table III), no significant associations were observed with any of the clinicopathological variables. When examining malignant tumors and NF- $\kappa$ B (p65) expression, tumor grade, perineural invasion, lymphovascular invasion, laterality, type of biopsy and tumor location, no significant associations were observed. Notably, sex and TNM stage were significantly associated with NF- $\kappa$ B (p65) expression (Table IV).

*Diagnostic performance of NF- $\kappa$ B (p65) as a marker in HNTs in CCTH.* The diagnostic performance of NF- $\kappa$ B (p65) protein expression in HNTs was determined by drawing receiver operating characteristic curves with X1stat. Sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values were determined and the results are presented in Fig. 2 and Table V. The overall sensitivity was 62.5%, with a specificity of 68.8%. The PPV and NPV were 60.0 and 71.1% respectively. Using a cut-off value  $\geq 0.600$ , the area under the curve was calculated as 0.656 ( $P<0.0001$ ).

## Discussion

A number of inflammatory mediators and signaling pathways have been linked to the development of HNCs (22). However, NF- $\kappa$ B, a key driver and inducer of inflammatory mediators that promote cell survival and therapeutic resistance (23), has not been extensively investigated in numerous African countries, including Ghana. Additionally, there is a global paucity

Table III. Association between NF-κB (p65) expression status and benign head and neck tumors.

Variable, n (%)	Total (n=62)	NF-κB (p65) expression status		χ <sup>2</sup> /Fisher's	P-value
		Low (n=44)	High (n=18)		
Sex				0.677	0.572
Male	26 (41.9)	17 (65.4)	9 (34.6)		
Female	36 (58.1)	27 (75.0)	9 (25.0)		
Age group, years				0.389 <sup>a</sup>	0.899 <sup>a</sup>
1-29	20 (32.3)	15 (34.1)	5 (27.8)		
30-57	38 (61.3)	26 (59.1)	12 (66.7)		
58-86	4 (6.4)	3 (6.8)	1 (5.6)		
Type of biopsy				3.201 <sup>a</sup>	0.332 <sup>a</sup>
Excision	40 (64.5)	29 (72.5)	11 (27.5)		
Incision biopsy	18 (29.0)	12 (66.7)	6 (33.3)		
Punch biopsy	3 (4.8)	3 (100.0)	0 (0)		
Free needle core biopsy	1 (1.6)	0 (0)	1 (100.0)		
Tumor location				10.900 <sup>a</sup>	0.302 <sup>a</sup>
Ear	5 (8.1)	4 (80.0)	1 (20.0)		
Oral cavity	6 (9.7)	4 (66.7)	2 (33.3)		
Salivary	2 (3.2)	1 (50.0)	1 (50.0)		
Eye	4 (6.5)	3 (75.0)	1 (25.0)		
Facial	7 (11.3)	7 (100.0)	0 (0)		
Larynx	5 (8.1)	3 (60.0)	2 (40.0)		
Mandible	8 (12.9)	5 (62.5)	3 (37.5)		
Maxillary	4 (6.5)	2 (50.0)	2 (50.0)		
Nasal cavity	16 (25.8)	13 (81.3)	3 (18.8)		
Nasopharynx	2 (3.2)	0 (0)	2 (100.0)		
Neck	3 (4.8)	2 (66.7)	1 (33.3)		
Laterality				0.642 <sup>a</sup>	0.727 <sup>a</sup>
Left	17 (27.4)	12 (70.6)	5 (29.4)		
Right	17 (27.4)	11 (64.7)	6 (35.3)		
N/A	28 (45.2)	21 (75.0)	7 (25.0)		

Data were analyzed using the Chi-squared test or <sup>a</sup>Fisher's exact test. N/A, not applicable.

of data on this topic. Therefore, the present study was designed to provide pertinent information on NF-κB (p65) expression and its association with the clinicopathological characteristics of patients with HNTs.

In the present study, only cytoplasmic immunostaining was observed in all the HNTs examined. Consistent with the findings of the present study, cytoplasmic NF-κB (p65) has been reported as a good prognostic marker in triple-negative breast cancer (24). Moreover, Al-Mutairi and Habashy (25) demonstrated that cytoplasmic NF-κB (p65) expression was associated with tumor size and high grade. Furthermore, Barnes *et al* (20) demonstrated that cytoplasmic NF-κB (p65) was linked to tumor grade. Conversely, NF-κB (p65) expression has been found in the nucleus (26). The findings of the present study suggest that NF-κB (p65) may be restrained in the cytosol, preventing its nuclear translocation and subsequent transcriptional regulation through DNA binding. This finding indicates the dysfunction of NF-κB (p65) signaling in HNTs, which may provide insight into the pathophysiology of these

cancers and may highlight its potential for use as a prognostic biomarker and therapeutic target.

One of the main findings of the present study was the substantial association between sex and NF-κB (p65) expression in HNC. According to Pasquali *et al* (27), the tumor microenvironment involving NF-κB is influenced by hormonal and inflammatory dynamics, which may vary by sex. This suggests that sex hormones have the potential to modulate NF-κB (p65) events. Moreover, the data of the present study revealed that males expressed higher levels of NF-κB (p65) than females, with a statistically significant difference (P=0.013). This notable sex difference in NF-κB (p65) expression levels may have significant clinical ramifications, influencing tumor behavior and therapeutic responses. It also indicates that NF-κB (p65) regulation in these tumors may be influenced by sex-specific differences in the immune response and inflammation. Generally, it is well-established that females mount stronger immune responses and have chronic inflammation at lower levels compared to males (28).

Table IV. NF- $\kappa$ B (p65) status in malignant head and neck tumors.

Variable, n (%)	Total (n=50)	NF- $\kappa$ B (p65) status		$\chi^2$ /Fisher's	P-value
		Low (n=20)	High (n=30)		
Sex				5.556	<b>0.038</b>
Male	30 (60.0)	8 (40.0)	22 (73.3)		
Female	20 (40.0)	12 (60.0)	8 (26.7)		
Age group, years				0.420 <sup>a</sup>	0.551 <sup>a</sup>
1-29	7 (14.0)	2 (10.0)	5 (16.7)		
30-57	20 (40.0)	8 (40.0)	12 (40.0)		
58-86	23 (46.0)	10 (50.0)	13 (43.7)		
Type of biopsy				4.361 <sup>a</sup>	0.516 <sup>a</sup>
Excision	14 (28.0)	5 (25.0)	9 (30.0)		
Incision biopsy	28 (56.0)	12 (60.0)	16 (53.3)		
Punch biopsy	5 (10.0)	1 (5.0)	4 (13.3)		
Free needle core biopsy	1 (2.0)	1 (5.0)	0 (0)		
Curettage	1 (2.0)	1 (5.0)	0 (0)		
Ultrasound guided core biopsy	1 (2.0)	0 (0)	1 (3.3)		
Tumor location				5.177 <sup>a</sup>	0.928 <sup>a</sup>
Ear	1 (2.0)	1 (5.0)	0 (0)		
Oral cavity	4 (8.0)	1 (5.0)	3 (10.0)		
Salivary	5 (10.0)	2 (10.0)	3 (10.0)		
Eye	1 (2.0)	1 (5.0)	0 (0)		
Larynx	11 (22.0)	4 (20.0)	7 (23.3)		
Mandible	1 (2.0)	1 (5.0)	0 (0)		
Maxillary	3 (6.0)	1 (5.0)	2 (6.7)		
Nasal cavity	8 (16)	3 (15.0)	5 (16.7)		
Nasopharynx	11 (22)	4 (20.0)	7 (23.3)		
Neck	5 (10.0)	2 (10.0)	3 (10.0)		
Tumor grade				0.940	0.828
Well	15 (30.0)	6 (30.0)	9 (30.0)		
Moderate	16 (32.0)	5 (25.0)	11 (36.7)		
Poor	19 (38.0)	9 (45.0)	10 (33.3)		
PNI				1.531	0.216
Present	1 (2.0)	1(5.0)	0 (0)		
Absent	49 (98.0)	19 (95)	30 (100.0)		
LVI				0.000	0.999
Present	5 (10.0)	2 (10.0)	3 (10.0)		
Absent	45 (90.0)	18 (90.0)	27 (90.0)		
Laterality				3.734	0.156
Left	15 (30.0)	9 (45.0)	6 (20.0)		
Right	11 (22.0)	4 (20.0)	7 (23.3)		
N/A	24 (48.0)	7 (35.0)	17 (56.7)		
TNM stage				6.551	<b>0.010</b>
I-II	33 (66.0)	9 (45.0)	24 (80.0)		
III-IV	17 (34.0)	11 (55.0)	6 (20.0)		

Data were analyzed using the Chi-squared test or <sup>a</sup>Fisher's exact test. Values in bold font indicate statistically significant differences (P<0.05). N/A, not applicable; PNI, perineural invasion; LVI, lymphovascular invasion; TNM, tumor node metastasis.

The exact mechanism(s) underpinning this link is unknown; thus, further investigations are required to clarify the

pathogenesis and hormonal elements causing this sex-based difference. The analysis of malignant tumors revealed that

Table V. Diagnostic performance of NF-κB (p65) in head and neck tumors.

Protein	Cut-off value	Sensitivity (%) 95% (CI)	Specificity (%) 95% (CI)	PPV (%) (49.5-69.6)	NPV (%) (62.1-78.5)	AUC (0.57-0.75)	P-value
NF-κB (p65)	0.600	62.5 (48.374.8)	68.8 (56.5-78.8)	60.0 (49.5-69.6)	71.0 (62.1-78.5)	0.656 (0.57-0.75)	P<0.0001

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

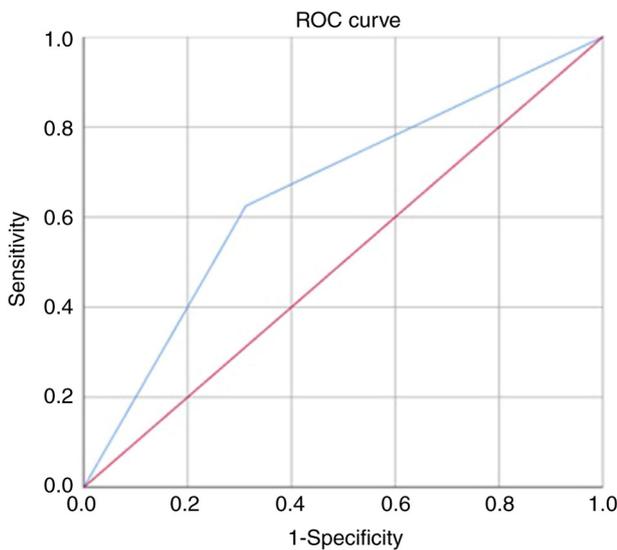


Figure 2. Receiver operator characteristic curve evaluation of NF-κB (p65) sensitivity and specificity among head and neck tumors. The sensitivity, specificity and AUC values are presented in Table V.

sex and TNM stage were significantly associated with NF-κB (p65) protein expression. This is consistent with the findings of other studies on other types of cancer, where NF-κB (p65) was shown to be significantly associated with TNM stage (29,30). The sex-specific association observed in malignant tumors underscores the importance of considering sex-related factors, such as hormonal receptor expressions in future studies, on NF-κB (p65) and HNC. These findings also suggest that sex-specific therapeutic approaches should be explored when targeting NF-κB (p65)-related pathways in the treatment of HNC. Furthermore, the association between NF-κB (p65) protein expression and TNM stage in malignant tumors highlights the critical role of NF-κB (p65) signaling in cancer progression and metastasis. Elevated NF-κB (p65) levels are typically linked to more advanced disease stages and poorer clinical outcomes.

Another main result of the present study was the association between NF-κB (p65) protein expression and tumor types (benign vs. malignant). Malignant tumors expressed significantly high NF-κB (p65) similar to previous studies (20,25). This finding raises the possibility that the NF-κB (p65) may be involved in the pathophysiology of malignant transformation in head and neck cancer. Numerous pro-tumorigenic mechanisms, such as inflammation, cell survival and proliferation, have been connected to NF-κB (p65) activation. Therefore, its

increased expression in malignant tumors may indicate a role in promoting tumor growth.

The present study found no significant links between NF-κB (p65) protein expression and age, biopsy type, tumor site, laterality (in both benign and malignant tumors), tumor grade, perineural invasion and lymphovascular invasion in malignant tumors. However, while age and declining immunity has been shown to be associated with NF-κB (31), similarly, the present study found a higher expression of NF-κB (p65) among older populations. However, the results of the present study may have been influenced by its retrospective design, limited clinicopathological variables analyzed and immunohistochemistry scoring methods.

The overall diagnostic performance of NF-κB (p65) in distinguishing between benign and malignant HNTs was moderate. The moderate sensitivity and specificity indicates that while NF-κB (p65) may not be sufficient as a stand-alone prognostic tool, it could be combined with other markers or clinical data to enhance predictive value and improve patient prognosis. To establish NF-κB (p65) as a reliable prognostic marker for HNTs, additional studies with larger and more diverse patient populations are required. Further studies are required to better determine the factors influencing NF-κB (p65) expression and refine its utility as a diagnostic and a prognostic tool.

In conclusion, the present study emphasizes that NF-κB (p65) expression levels may indeed be controlled by sex and TNM stage, with males usually exhibiting a higher NF-κB (p65) activity than females in HNC. These results highlight the need for further studies to elucidate the molecular mechanisms regulating NF-κB (p65) expression, advanced disease stages and sex disparities, which may involve genetic, hormonal and environmental factors. Investigating its potential as a prognostic biomarker and therapeutic target is crucial to improving the outcomes and wellbeing of patients.

**Acknowledgements**

The authors would like to thank Mr. Samuel Mingyigilougu Apewe Ka-Chungu of the Department of Pathology at Komfo Anokye Teaching Hospital, Kumasi, Ghana for assisting with tissue sectioning.

**Funding**

The Directorate of Research, Innovation and Consultancy (RSG/GRP/CoHAS/2022/104), University of Cape Coast (Cape Coast, Ghana) funded this study with additional support

from Samuel and Emelia Brew-Butler/Graduate Studies research grant, University of Cape Coast.

### Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

### Authors' contributions

PB and ROS were involved in the conceptualization and design of the study, as well as in data collection, data analysis, project administration, manuscript writing and funding acquisition. AM, LDK, EAd, EAg, ESY, BA, EGI, GA, PKA, FHL, KD, SVN, DOY were involved in the laboratory investigation, literature review, data analysis and the drafting of the manuscript. All authors have read, edited and approved the final manuscript. PB and EAd confirm the authenticity of the raw data.

### Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Cape Coast Teaching Hospital (reference no. CCTHERC/EC/2023/183). Consent to participate was waived as it was a retrospective study which did not violate patient privacy.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

### References

- Sarwar S, Mulla M, Mulla M, Tanveer R, Sabir M, Sultan A and Malik SA: Human papillomavirus, tobacco, and poor oral hygiene can act synergetically, modulate the expression of the nuclear factor kappa B signaling pathway for the development and progression of head and neck cancer in the Pakistani population. *Chin Med J (Engl)* 135: 1829-1836, 2022.
- D'cruz A, Lin T, Anand AK, Atmakusuma D, Calaguas MJ, Chitapanarux I, Cho BC, Goh BC, Guo Y, Hsieh WS, *et al*: Consensus recommendations for management of head and neck cancer in Asian countries: A review of international guidelines. *Oral Oncol* 49: 872-877, 2013.
- Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H and Miyazaki T: A review of HPV-related head and neck cancer. *J Clin Med* 7: 241, 2018.
- Leemans CR, Braakhuis BJ and Brakenhoff RH: Response to correspondence on the molecular biology of head and neck cancer. *Nat Rev Cancer* 11: 382-382, 2011.
- Adeyi A and Olugbenga S: The challenges of managing malignant head and neck tumors in a tropical tertiary health center in Nigeria. *Pan Afr Med J* 10: 31, 2011.
- Cramer JD, Burtneess B, Le QT and Ferris RL: The changing therapeutic landscape of head and neck cancer. *Nat Rev Clin Oncol* 16: 669-683, 2019.
- Alterio D, Marvaso G, Ferrari A, Volpe S, Orecchia R and Jereczek-Fossa BA: Modern radiotherapy for head and neck cancer. *Semin Oncol* 46: 233-245, 2019.
- Owusu-Afriyie O, Owiredu WKBA, Owusu-Danquah K, Komarck C, Foltin SK, Larsen-Reindorf R, Acheampong E, Quayson SE, Prince MEP, McHugh JB, *et al*: Expression of immunohistochemical markers in non-oro-pharyngeal head and neck squamous cell carcinoma in Ghana. *PLoS One* 13: e0202790, 2018.
- Barnes P, Yeboah F, Zhu J, Saahene RO, Akakpo P and Ephraim RK: Prognostic significance of epidermal growth factor receptor (EGFR) in head and neck tumours at some selected hospital in Ghana, 2019.
- Barnes P, Yeboah FA, Zhu J, Saahene RO, Obirikorang C, Adinortey MB, Amoani B, Kyei F, Akakpo P and Awuku YA: Prognostic worth of epidermal growth factor receptor (EGFR) in patients with head and neck tumors. *J Cancer Epidemiol* 2020: 5615303, 2020.
- Morgan EL, Chen Z and Van Waes C: Regulation of NF $\kappa$ B signalling by ubiquitination: A potential therapeutic target in head and neck squamous cell carcinoma? *Cancers (Basel)* 12: 2877, 2020.
- Taniguchi K and Karin M: NF- $\kappa$ B, inflammation, immunity and cancer: Coming of age. *Nat Rev Immunol* 18: 309-324, 2018.
- Perkins ND: The diverse and complex roles of NF- $\kappa$ B subunits in cancer. *Nat Rev Cancer* 12: 121-132, 2012.
- El-Rayes BF, Ali S, Ali IF, Philip PA, Abbruzzese J and Sarkar FH: Potentiation of the effect of erlotinib by genistein in pancreatic cancer: The role of Akt and nuclear factor-kappaB. *Cancer Res* 66: 10553-10559, 2006.
- Biswas DK, Shi Q, Baily S, Strickland I, Ghosh S, Pardee AB and Iglehart JD: NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis. *Proc Natl Acad Sci USA* 10137-10142, 2004.
- Kim HJ, Hawke N and Baldwin AS: NF-kappa B and IKK as therapeutic targets in cancer. *Cell Death Differ* 13: 738-747, 2006.
- Terzić J, Grivennikov S, Karin E and Karin M: Inflammation and colon cancer. *Gastroenterology* 138: 2101-2114.e5, 2010.
- Li CW, Xia W, Huo L, Lim SO, Wu Y, Hsu JL, Chao CH, Yamaguchi H, Yang NK, Ding Q, *et al*: Epithelial-mesenchymal transition induced by TNF- $\alpha$  requires NF- $\kappa$ B-mediated transcriptional upregulation of Twist1. *Cancer Res* 72: 1290-1300, 2012.
- Chou YC, Sheu JR, Chung CL, Chen CY, Lin FL, Hsu MJ, Kuo YH and Hsiao G: Nuclear-targeted inhibition of NF-kappaB on MMP-9 production by N-2-(4-bromophenyl) ethyl caffeamide in human monocytic cells. *Chem Biol Interact* 184: 403-412, 2010.
- Barnes P, Mensah A, Derkyi-Kwarteng L, Adankwa E, Agbo E, Yahaya ES, Amoani B, Adjei G, Ka-Chungu SMA, Akakpo PK, *et al*: Prognostic significance of nuclear factor kappa B (p65) among breast cancer patients in cape coast teaching hospital. *Med Princ Pract* 33: 1-11, 2024.
- Adankwah E, Danquah K, Gyamfi D, Sampene P, Ossei P and Asiamah E: Nuclear localisation of autophagic p62 and associated cytoplasmic Beclin-1 and Bcl-2 expressions in adenomas and adenocarcinomas of the colorectal regions. *J Carcinog Mutagen* 9: 2, 2018.
- Yang X, Cheng H, Chen J, Wang R, Saleh A, Si H, Lee S, Guven-Maiorov E, Keskin O, Gursoy A, *et al*: Head and neck cancers promote an inflammatory transcriptome through coactivation of classic and alternative NF- $\kappa$ B pathways. *Cancer Immunol Res* 7: 1760-1774, 2019.
- Xia Y, Shen S and Verma IM: NF- $\kappa$ B, an active player in human cancers. *Cancer Immunol Res* 2: 823-830, 2014.
- Baba M, Takahashi M, Yamashiro K, Yokoo H, Fukai M, Sato M, Hosoda M, Kamiyama T, Taketomi A and Yamashita H: Strong cytoplasmic expression of NF- $\kappa$ B/p65 correlates with a good prognosis in patients with triple-negative breast cancer. *Surgery Today* 46: 843-851, 2016.
- Al-Mutairi MS and Habashy HO: Nuclear factor- $\kappa$ B clinical significance in breast cancer: An immunohistochemical study. *Med Princ Pract* 32: 33-39, 2023.
- Yan M, Xu Q, Zhang P, Zhou XJ, Zhang ZY and Chen WT: Correlation of NF-kappaB signal pathway with tumor metastasis of human head and neck squamous cell carcinoma. *BMC Cancer* 10: 437, 2010.

27. Pasquali D, Giacomelli L, Pedicillo MC, Conzo G, Gentile G, De Stefano IS, Angelillis F, Santoro A, Miele F, Digitale Selvaggio L, *et al*: Tumor inflammatory microenvironment of the thyroid cancer: Relationship between regulatory T-Cell imbalance, and p-NFKB (p65) Expression-A preliminary study. *J Clin Med* 12: 6817, 2023.
28. Roved J, Westerdahl H and Hasselquist D: Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. *Horm Behav* 88: 95-105, 2017.
29. Zhou XL, Fan W, Yang G and Yu MX: The clinical significance of PR, ER, NF- $\kappa$ B, and TNF- $\alpha$  in breast cancer. *Dis Markers* 2014: 494581, 2014.
30. Pyo JS and Kim EK: Clinicopathological significance and prognostic implication of nuclear factor- $\kappa$ B activation in colorectal cancer. *Pathol Res Pract* 215: 152469, 2019.
31. Songkiatissak PS, Rahman MT, Aqdas M and Sung MH: NF- $\kappa$ B, a culprit of both inflamm-ageing and declining immunity? *Immun Ageing* 19: 20, 2022.



Copyright © 2025 Barnes et al. This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) License.