Original Article

Proinflammatory Cytokine Profile in Head and Neck Squamous Cell Carcinoma Patients: Preliminary Report

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Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignant neoplasms globally. Tobacco and alcohol use are predominant risk factors. Inflammation plays a major role in the etiopathogenesis of cancers and cytokines influence activation, growth, and differentiation of several target cells. This study aimed at profiling head and neck cancer patients with regards to the serum level of proinflammatory cytokines and disease stage. **Materials and Methods:** This study involved thirty newly diagnosed cases of HNSCC and thirty apparently healthy comparable group. Five milliliter of venous blood was collected from the antecubital vein of each individual. The blood samples dispensed in plain bottles were allowed to clot and retract; serum was stored at −20°C until analysis. Cytokine assay was carried out by enzyme-linked immunosorbent assay technique using a commercial kit. **Results:** The study group was made up of 19 (63.3%) males and 11 (36.7%) females. The ages ranged from 19 to 92 years with a mean of 59.1 ± 19.1 years. The comparative group was made up of 14 (46.7%) males and 16 (53.3%) females with a mean age of 63.2 ± 7.5 years. The oral cavity was the most common site of SCC [6 (20.0%)]. The cases were mostly advanced in 28 (93.3%, Stages III and IV). Serum interleukin-1 IL-1 (β), IL-6, IL-8, TNF – α, and IFN – γ were elevated in the test cases relative to the controls. This however, was only statistically significantly so with IL-6 and IL-8. The mean values for both cytokines for all tumor sites were exceeded in nasopharyngeal carcinoma. **Conclusion:** Serum IL-6 and IL-8 appear to be significant in the pathology of head and neck cancer and could be explored for possible roles in the management of HNSCC.

Keywords: Carcinoma, proinflammatory, cytokines head neck

Introduction

Head and neck cancers represent the sixth most common cancer worldwide, with approximately 630,000 new patients diagnosed annually resulting in >350,000 deaths every year.[1] More than 90% of head and neck cancers are squamous cell carcinomas (HNSCCs) that arise from the mucosal surfaces of the oral cavity (ICD-10 code: C00-08), oropharynx (ICD-10 code: C09-10 and C12-14), and larynx (ICD-10 code: C32-9).[2] There is wide geographical variation in the incidence and anatomic distribution of HNSCC worldwide; this is predominately attributable to demographic differences in the habits of tobacco and alcohol use.[1] Although the mechanisms driving tissue transformation that result in malignant transformation are incompletely understood, these etiologic factors contribute to carcinogenesis in several ways including genotoxicity and increased proinflammation and proangiogenesis cytokine expression and aberrant pathway signaling.[3-5] Cytokines are typically divided into two categories: proinflammatory (e.g., IL-1 (β), IL-6, IL-8, TNF-α [IFN-γ]) and anti-inflammatory (e.g., IL-4, IL-10, and TGF-(β), and VEGF).[6] These factors modulate tissue remodeling and angiogenesis and actively promote tumor cell survival and chemoresistance through autocrine and paracrine mechanisms.[7] Altered expression of cytokines and growth factors plays a major role in the malignant transformation of many cancers including HNSCC.[8,9] The aim of this study was to carry out a cytokine profiling of patients with head and neck cancer and relate the same with the disease stage.

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Materials and Methods

This was a cross-sectional study conducted at a tertiary center in Southwest, Nigeria. It involved thirty newly diagnosed cases of HNSCC and thirty apparently healthy volunteers who served as a comparative group. Patients with ongoing infection received appropriate treatment, while others with comorbidities were referred for management before commencement of the study. Five milliliter of venous blood was collected from the antecubital vein without venous stasis from each individual. The blood sample dispensed in a plain bottle was allowed to clot and retract after which the serum was separated into a plain cryovial bottle for storage and subsequent analysis. The specimen was stored at −20°C until time of analysis.

Analysis for cytokines

Concentrations of IL-1(β), IL-6, IL-8, TNF-a, and IFN-γ in serum were analyzed with the enzyme-linked immunosorbent assay (ELISA)-based technique using a commercially available ELISA kit by indirect sandwich technique (RayBiotech R and D Systems, GA) according to the manufacturer's instruction. Cytokine concentrations were obtained by interpolation of the standard curve. Standard curves were generated for each analyte and sample concentrations were calculated from the standard curve. Select samples were done in duplicates, thereby serving as intraassay controls. Data were analyzed using descriptive statistic with level of significance set at $P < 0.05$.

Results

The study group was made up of thirty recently diagnosed cases of HNSCC, consisting of 19 (63.3%) males and 11 (36.7%) females aged between 19 and 92 years. Thirty apparently healthy individuals aged between 21 and 76 years, made up of 14 (46.7%) males and 16 (53.3%) females, served as comparative group. The mean ages of the cases and controls were, however, 59 (standard deviation [SD] ± 19.1) and 63 (SD ± 7.5) years, respectively. There was a male dominance in the incidence of the cancers at a male-to-female ratio of 1.7: 1 [Table 1]. The oral cavity was the most common site for HNSCC, making up 20% of all cases [Table 2]. Majority 28 (93.3%) of cases were advanced disease; Stages III and IV. No Stage I disease was recorded. The serum levels of all cytokines were higher in cases compared to comparative group; however, it was only significantly so with IL-6 and IL-8 [Table 3]. Among the studied risk factors, only tobacco–alcohol combination showed significant association with cancer ($P = 0.00$) [Table 4]. Nasopharyngeal tumors had serum values of IL-6 and IL-8 higher than mean for all tumor sites.

Table 1: Frequency/Percentages

Characteristics	Cases	Control
Gender		
Male	19 (63.3)	20 (66.7)
Female	11 (36.7)	10 (33.3)
Age		
<40	9 (30.0)	1 (3.3)
40-49	4 (13.3)	2 (6.7)
50-59	5 (16.7)	3 (10)
59-69	5 (16.7)	16 (53.3)
≥70	7 (23.3)	8((26.7)
Total	30 (100)	30 (100)

Table 2: Site and frequency distribution of tumours

Site of tumour	Frequency	Percentage
Antrum	4	13.3
Oral cavity	6	20
Larynx	1	3.3
Mandible	5	16.7
Nasal cavity	1	3.3
Nasopharynx	5	16.7
Orbital mass	1	3.3
Infra-auricular area	1	3.3
Neck (NOS)	5	16.7
Vocal cord	1	3.3
Total	30	100

*NOS: Not otherwise specified (squamous cell carcinoma)

Discussion

In addition to the genetic changes that are recognized as the hallmarks of cancer, other processes involving cells within the tumor microenvironment that are not necessarily cancerous themselves are significant.[9] Cancer-related inflammation is considered the "seventh hallmark of cancer;" several studies have demonstrated that tumors develop and progress as inflammatory diseases.[10] Molecules including cytokines play key roles in both inflammation and cancer by promoting proliferation, angiogenesis, and carcinogenesis and by recruiting immune cells.[11] HNSCC cells have been shown to express both IL-6 and its receptors.[12] Our results showing elevated serum levels of IL-6 in SCC patients are in accordance with the previous studies.[13,14] Current evidence suggests that IL-6 can promote tumor cell proliferation in several tumor cell lines including HNSCC,[12] and patients with higher IL-6 serum levels have been shown with more advanced tumor.[13]

In general, a tumor-promoting role for IL-8 has been identified in a wide variety of human solid tumors including malignant melanoma; non-small cell lung cancer; malignant mesothelioma; head and neck squamous carcinoma; cervical and endometrial carcinoma; epithelial ovarian carcinoma; gastric, pancreatic, and colorectal carcinomas; hepatocellular carcinoma; androgen-independent prostate adenocarcinoma; renal cell carcinoma; breast cancer; and Kaposi's sarcomas.[15-28] IL-8 production is linked with tumor vascularization, metastatic phenotype, tumor growth, and overall poor prognosis.[29] Our results showing elevated IL-8 levels in HNSCC patients are in concordance with previous studies demonstrating elevated serum concentrations of IL-8

Table 3: Comparison of mean cytokine values between cases and controls

	Mean (SD)	Median	IQR	*P*
TNF				
Case	259.7 (201.7)	200.3	130.4	0.52
Control	233.3 (101.1)	200.3	116.5	
IL1				
Case	5.6 (12.8)	3.1	0.6	0.34
Control	3.4 (0.4)	3.4	0.4	
IFN				
Case	1983.6 (2002.3)	1524.8	42.1	0.37
Control	1641.5 (522.1)	1519.5	47.9	
IL6				
Case	335.37 (302.5)	257.5	26.2	0.05
Control	253.5 (11.6)	260.1	22.0	
IL8				
Case	315.9 (535.2)	83.4	170.9	0.00
Control	47.5 (42.4)	30.5	39.3	

Table 4: Comparisons between Risk Factors in Cases and Control

Variable	Test *n* (%)	Control *n* (%)	Total *n* (%)	$t\ \chi^2$	*P*
Family history of cancer					
None	27 (91.0)	26 (86.7)	53 (88.0)	2.66	0.80
Parents	3 (0.9)	0 (0.0)	3 (4.0)		
Sibling	0 (0.0)	2 (6.7)	2 (4.0)		
Children	0 (0.0)	2 (6.7)	2 (4.0)		
Total *n* (%)	30 (100)	30 (100)	60 (100)		
Tobacco-alcohol use					
Yes	19 (64.0)	2 (6.7)	21 (28.8)		0.00*
No	11 (36.0)	28 (93.3)	39 (72.0)		
Total *n* (%)	30 (100)	30 (100)	60 (100)		
Alcohol intake					
Yes	16 (55.0)	18 (60.0)	34 (56.0)	0.24	0.7
No	14 (45.0)	12 (40.0)	26 (44.0)		
Total *n* (%)	30 (100)	30 (100)	60 (100)		

in HNSCC patients.[29,30] Our findings also support the fact that HNSCCs are highly inflammatory in nature as previously reported, and this may contribute to the rather relatively aggressive nature of the disease.[7] It is also plausible to say that nasopharyngeal tumors may be more inflammatory, in view of the serum values of IL-6 and IL-8 being higher in these patients than the mean in all tumor sites.

Conclusion

We conclude that IL-6 and IL-8 may play an important role in HNSCC tumorigenesis. However, further studies on a larger patient population will be needed to evaluate the association between serum cytokine levels and disease stage as well as the potential use of these cytokines as biomarkers in head and neck cancer. It also has to be noted that serum cytokine profiles might be influenced by processes independent of head and neck cancer; therefore, it might be necessary to combine serum cytokine profiles with other markers, for more sensitive and specific interpretations. Furthermore, the observed increases in IL-6 and IL-8 could not be correlated with disease stage, as cases were mainly advanced at presentation. Although Kim *et al*. have demonstrated that serum levels of cytokines do not differ significantly between healthy young adults and elderly individuals, appropriate matching of test and control is desirable.[31] In addition, values obtained from the cases and the comparative group could also not be related to a reference range because of the reported huge variations, although values could be extrapolated.

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Conflicts of interest

There are no conflicts of interest.

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